Method for Loading Amphiphile Particles with Active Agents

The present invention relates to methods for the
production of particles suitable for the delivery of
active substances. More specifically, the invention
relates to methods for the production of amphiphile-
based particles containing a high level of active agent.

Amphiphile-based formulations show considerable
potential in the delivery of many substances, especially
for *in vivo* delivery to the human or animal body.
Because the amphiphile has both polar and apolar groups
which cluster to form polar and apolar regions, it can
effectively solubilise both polar and apolar compounds.
In addition, many of the structures formed by
amphiphiles/structuring agents in polar and/or apolar
solvents have a very considerable area of polar/apolar
boundary at which other amphiphilic compounds can be
adsorbed and stabilised. Amphiphile compositions are
thus highly suitable for the delivery of compounds of
limited aqueous solubility.

The formation of lamellar, non-lamellar and micellar
regions in the amphiphile/water, amphiphile/oil and
amphiphile/oil/water phase diagrams is a well known
phenomenon. Non-lamellar phases include liquid
crystalline phases such as the cubic P, cubic D, cubic G
and hexagonal phases, which are fluid at the molecular
level but show significant long-range order, and the L_3
"sponge" phase which comprises a multiply interconnected
three-dimensional bi-continuous network of bilayer
sheets which lack the long-range order of the liquid
crystalline phases. Lamellar phases take the form of
bilayer sheets which close to form uni- or multi-
lamellar vesicles or liposomes and micellar phases adopt

clusters of amphiphile molecules having one group (polar
or apolar) directed towards a continuous region and the
other group (apolar or polar) directed towards the
centre of the cluster.

Depending upon their curvature, the various phases may
be described as normal (mean curvature towards the
apolar region) or reversed (mean curvature towards the
polar region). Where the spontaneous curvature of the
lipid system is close to zero, the structures are
typically lamellar, such as uni- or multi-lamellar
vesicles/liposomes and where the spontaneous curvature
is more negative or positive, cubic, hexagonal and
micellar phases typically dominate.

The various phases including micellar, lamellar and non-
lamellar (e.g. liquid crystalline and L_3 phases) are
thermodynamically stable systems. That is to say, under
suitable conditions they are not simply a meta-stable
state that will separate and/or reform into layers, or
other phases, but are the thermodynamically stable form
of the mixture.

Lamellar, non-lamellar and micellar systems have all
been investigated for their properties as carriers
and/or excipients for dietary, cosmetic, nutritional,
diagnostic and pharmaceutical agents. All phase
structures are of value under certain circumstances, the
most appropriate depending upon the specific
application. Non-lamellar systems in particular are
thought to have considerable advantages in terms of
their high internal surface area between polar and
apolar regions. This has led to considerable
investigation of amphiphilic phase structures in
controlled-release formulations and for solubilising
compounds of relatively low solubility.

As discussed above, a bulk non-lamellar phase is
typically a thermodynamically stable system, as are
certain dispersed phases such as micelles and vesicles.
In addition, the bulk non-lamellar phase may be
dispersed in a polar or non-polar solvent to form
particles of a non-lamellar (especially liquid
crystalline) phase in a bulk solvent. The particles are
thus reminiscent of micelles or vesicles but contain a
non-lamellar structured core region. Such non-lamellar
dispersions allow the advantages of bulk non-lamellar
phases to be applied in situations where use of a bulk
non-miscible phase would cause problems, such as in
parenteral applications. Further control of a
compound's release profile may also be achieved by such
a dispersion of non-lamellar particles. For example,
the particles may be fully (i.e. thermodynamically)
stable, or may gradually degrade, thereby providing
control over the release profile for active agents
formulated therewith.

The formation of dispersions can be spontaneous or as
the result of mechanical force such as shearing or
ultrasound. Non-lamellar particles are of considerable
interest in the delivery of active agents and have been
proposed as carriers for many such actives.

A method for the formation of dispersed particles of
non-lamellar phase in solvents such as water is
described in US 5,531,925. Such particles have a non-
lamellar liquid crystalline or L_3 interior phase and a
lamellar or L_3 surface phase and may also contain active
ingredients. Methods for the formation of lamellar
vesicles and micelles are well known in the art.

Known particles of liquid crystalline or L_3 interior
phase may be formed by methods such as adding to this

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phase a solution of surface-phase forming agent,
stirring to form a coarse dispersion and fragmenting the
resulting mixture.

Cryo-Transmission Electron Microscopy (cryo-TEM) may be
used to examine the particle size and phase structure(s)
present in a dispersion. In addition, the presence of a
liquid crystalline phase in a prospective liquid
crystalline material may be examined by use of small-
angle X-ray diffraction (SAX), or Nuclear Magnetic
Resonance (NMR) spectroscopy studies. The sizes and
size distributions of the dispersed particles may be
examined by light scattering, particularly by use of
laser light scattering or diffraction instruments.

Dispersions containing active ingredients, and
particularly those for intravenous administration to the
human or animal body, are desirably colloidal. That is,
they should be of a particle size no greater than 10 μm,
especially no greater than 5 μm and particularly no
greater than 1 μm. If particles within the dispersion
exceed this size then the dispersion may not be
colloidally stable and there is a considerable risk of
causing embolism when the preparation is administered
intravenously. Furthermore, it is desirable that the
distribution of particle sizes be narrow to maximise
control over the release of any active agent. Where a
particulate composition is to be administered by a
method other than intravenously (e.g. orally,
intramuscularly, subcutaneously, rectally or by
inhalation), then the particles need not necessarily be
colloidal but it remains advantageous to provide a well
characterised and reproducible particle size
distribution in order to control the rate of
decomposition of the particles and/or release of the
active agents.

The particle size, phase behaviour and active agent
loading of a particulate composition should also be
stable to storage over a considerable period of time.
If the distribution of particle sizes changes
significantly then the effective transport rate for
composition (e.g. due to diffusion and rate of release
of any active agent) may be adversely affected.
Similarly, if the loading level or phase behaviour of a
composition alters upon storage then the rate of release
of active agent will be altered and control over the
release profile may be lost.

Of still greater concern is the stability of particle
sizes in a colloidal dispersion for intravenous
administration. If the particle size distribution of
such a dispersion is not stable (e.g. to storage and
distribution) then large particles may form over time
and be dangerous when administered. Even if not
directly dangerous, storage instability can cause
significant variability in pharmacokinetics, dynamics
and/or efficacy.

Known methods for the formation of dispersed particles
of lamellar, micellar and non-lamellar phase are highly
effective. Such dispersions are typically pre-formed
and subsequently loaded with active agent by
equilibrating the dispersion in a solution of active
agent.

In general, the level of active agent which can be
incorporated by equilibration in aqueous solution is
relatively low but has been assumed to be the maximum
level which could be stably retained by an amphiphilic
composition. To the extent that any additional loading
of active agent was even considered possible, it was
assumed that this would be greater than the particles

could accept when "saturated" and so would be highly
unstable.

Obviously, it would be a considerable advantage to be
able to load a greater proportion of active agent into
an amphiphilic composition. This would increase the
dose of active agent which could be delivered in a
particular administration volume, would give further
control over the release rate and would reduce the
quantity of excipient which must be formulated with the
active agent. This reduction would, in turn, reduce
possible undesirable effects where a lipid of limited
biotollerability was needed. Certain surfactants, for
example, produce toxic effects at high levels and have a
maximum acceptable daily intake of only a few mg per kg
body weight. Higher loading would also improve the
ease of production, transport and storage due to the
lower masses and volumes of excipient required.
Furthermore, smaller volumes may be administered in
vivo, which is desirable from a practical point of view
as well as potentially reducing discomfort on
administration (e.g. by injection)

In increasing the loading of an active agent, however,
it is highly desirable that the this active agent remain
stable in the dispersion and that the particle size and
phase behaviour remain stable and/or predictable.

It would thus be a considerable advantage to provide
amphiphile compositions, such as dispersions, having a
higher loading of active agent than can easily be
obtained by simple equilibration. It would be a further
distinct advantage if such dispersions were stable to
storage in terms of active agent loading, particle size
and/or phase behaviour.

The present inventors have now unexpectedly established
that by preparing a dispersion of amphiphile particles
of appropriate composition in a solution of active agent
and heating this dispersion to an elevated temperature
for a short period before cooling to around room
temperature, the level of active agent loading achieved
can be significantly greater than that obtainable by
simple equilibration. The present inventors have
furthermore established that such particles may be
stable to storage in terms of their active agent
loading, particle size and/or phase behaviour.

The present invention thus provides a method for the
production of (preferably colloidal) amphiphile based
particles having incorporated therein at least one
active agent, said method comprising forming a
dispersion of particles comprising at least one
amphiphilic structuring agent in a solution of at least
one active agent, heating said particles to an elevated
temperature, followed by cooling, preferably to around
ambient temperature. Generally, said heating will be to
a temperature and for a period sufficient to provide,
after cooling, an incorporation of active agent into
said particles which is at least 130% of the maximum
incorporation provided by equilibrating said particles
in a solution of at least one active agent at room
temperature or preferably 37°C for up to 3 days. This
heating and cooling method may be carried out once, or
as two, three, four or more sequential cycles of heating
and cooling.

The present invention further provides a method for
increasing the incorporation of at least one active
agent into (preferably colloidal) amphiphile particles,
above the level achievable by equilibration at room
temperature or preferably 37°C, said method comprising

forming a dispersion of particles comprising at least
one amphiphilic structuring agent in a solution of at
least one active agent, heating said particles to an
elevated temperature, followed by cooling, preferably to
around ambient temperature. Generally, said heating
will be to a temperature and for a period sufficient to
provide, after cooling, an incorporation of active agent
into said particles which is at least 130% of the
maximum incorporation provided by equilibrating said
particles in a solution of at least one active agent at
37°C (e.g. for up to 3 days). This heating and cooling
method may be carried out once, or as two, three, four
or more sequential cycles of heating and cooling.

Evidently, the particles and particle dispersions formed
by the methods of the invention have a greater level of
active agent incorporation than has previously been
achievable. Such particles and all dispersions, creams,
gels, powders and compositions thereof are thus new,
since these could not be made by previously known
methods.

In a further aspect, the present invention therefore
provides (preferably colloidal) amphiphile particles
comprising at least one structure forming amphiphile and
at least one active agent, wherein the incorporation of
active agent into said particles is at least 130% of the
maximum incorporation provided by incubating equivalent
particles not comprising any active agent in a solution
of at least one active agent at 37°C (e.g. for up to 3
days). The particles of the invention may be formed or
formable by the methods of the invention.

The heat cycling methods of the invention have
surprisingly general application and may be applied to
dispersions of many phase structures and for the loading

of many active agents. It is obviously desirable that
the active agent(s) be stable to the heat treatment
method and thus by "active agent" as used herein is
intended to indicate a bioactive agent being stable to
the heat treatment method and conditions to the extent
that no more than 50%, preferably no more than 20% and
most preferably no more than 10% of the active agent is
destroyed or rendered inactive by the chosen heat
treatment loading conditions (for example, those
described herein).

The particles of the present invention may be used as a
dispersion in a solvent (especially an aqueous solvent),
or may be dried and/or formulated as a pharmaceutical
composition.

In a further aspect, the present invention thus provides
a pharmaceutical composition comprising particles of the
present invention, optionally including at least one
pharmaceutically tolerable carrier or excipient.

In further aspects, the present invention also provides
powders and dispersions comprising particles of the
present invention, whether or not these are
pharmaceutical compositions and gels, creams, tablets,
capsules etc. incorporating such powders or dispersions.

The present invention provides methods by which
amphiphilic compositions may be loaded with more active
agent than could be achieved by equilibration. It is
the surprising finding of the inventors that this
elevated level of incorporation remains stable when the
particles are cooled (e.g. to 37°C, room temperature, 4°C
or even below). A most surprising aspect of the
invention, however, is that the high incorporation of
active agent provided by the methods described herein

remains stable to storage over a remarkably long period
of time.

It was the inventors' initial assumption that the
increased loading level observed by the present method
was the result of forming a metastable, "supersaturated"
type state and that consequently the particles formed
would not retain the active agent and would possibly be
unstable in terms of particle size and distribution over
a short period of time. It was therefore expected that
precipitation or separation of the active agent would be
observed or that the particle structure would be
disrupted to the extent that particles would break, fuse
or separate from the dispersion.

At present, no explanation can be offered as to why the
particles of and formed by the present invention are
stable, particularly in terms of size distribution and
to the loss of active agent. It may be, for example,
that a metastable "supersaturated" state with a long
lifetime is formed or that microscopic drops or crystals
of active agent become entrapped within the particles
and all of these possible embodiments fall within the
scope of the present invention. It is, however, the
observation of the inventors that a considerable number
of different amphiphile dispersions of varying
compositions and phase structures have remained stable
for many days with each of a number of active agent
incorporated at a level significantly in excess of that
achievable by other methods.

In a highly preferred aspect, the present invention thus
provides methods and particles of the invention wherein
the loaded particles are stable to the loss of active
agent for a period of no less than 24 hours at 25°C.
Preferably such particles are stable to the loss of

active agent when stored in aqueous dispersion at 25°C
for at least 5 days, more preferably at least 2 weeks
and most preferably at least 1 month. It is yet more
preferable that these periods of stability be shown upon
storage at 4°C, 25°C and 40°C. By "stable" to the loss
of active agent is meant that, at the end of the storage
period, no less than 90% and preferably no less than 95%
of the active agent remains incorporated into the
particles, relative to that present 1 hour after the (or
the last, if appropriate,) heating and cooling cycle.

The amphiphile based particles of the invention and
formed in the loading method of the invention are also
preferably stable to storage in terms of their size and
size distribution. Such formulations should be
essentially stable for periods of at least 24 hours at
25°C and generally 10 days at room temperature, more
typically at least 1 month, preferably at least 3 months
and more preferably 6 months or more. In contrast,
previously known dispersions of similar average particle
size even without the very high active agent loading
provided by the present invention may have particle
sizes stable for less than 10 days at room temperature.

In one embodiment, a particle size distribution can be
considered essentially stable to storage if the average
(mode or preferably mean) particle size increases no
more than two fold during the storage period.
Preferably, the average size should increase no more
than 50% and more preferably no more than 20% during the
storage period. Similarly, the width of the
distribution at half-height should preferably increase
by no more than 50%, more preferably by no more than 20%
and most preferably no more than 10% during the storage
period. Where a distribution is monomodal, it should
preferably remain monomodal during the storage period.

In a highly preferred embodiment, average particle size
and particle size distribution width at half-height
alter by no more than 10% and remain monomodal on
storage for the periods indicated above.

With colloidal formulations, especially for parenteral
use, a formulation may be considered stable to storage
if no more than 10%, preferably no more than 5%, of
particles (by volume) are larger than 10 µm, preferably
8 µm after the storage periods indicated above.

It is particularly important in the case of colloidal
dispersions for use in intravenous or intra-arterial
administration that the particle size distribution be
stable to storage. A composition containing even a
relatively small component of non-colloidal particles
may cause embolism, or at least unpredictable rates of
release upon administration directly to the blood
stream. Similarly, the controlled release of an active
agent may be dependent not only upon stable loading but
also upon a reliable particle size distribution in a
composition. Pharmaceutical, diagnostic and veterinary
products are also desirably stable to storage for
several months or the cost and availability of the
product is significantly adversely affected. The method
of the invention thus significantly improves the
prospect of an active agent formulated in a dispersion
of non-lamellar particles forming a safe and available
product with high effectiveness.

The method of the present invention may be used to load
suitable active agents into dispersed particles having
any formable phase structure (by choice of appropriate
compositions). It is preferable, however, that the
particles to be loaded are substantially not lamellar
particles (i.e. are substantially non-lamellar or

micellar) since a greater increase in loading has been
observed in such systems. More preferably, the
particles to be loaded with (at least one) active agent
will substantially be non-lamellar particles such as
particles of hexagonal or bicontinuous cubic phase.

In a preferred aspect of the invention, at least 75% (by
volume) of the particles to be loaded by the method of
the invention will be non-lamellar or micellar
(preferably non-lamellar). More preferably, at least
85% and most preferably at least 95% of particles to be
loaded will be non-lamellar or micellar (preferably non-
lamellar), as measured by volume. This measurement may
be made by, for example, laser diffraction, preferably
combined with cryo-TEM or SAXS (to confirm the particle
structure).

As used herein, the term "non-lamellar" is used to
indicate a normal or reversed liquid crystal phase (such
as a cubic or hexagonal phase) or the L_3 phase or any
combination thereof. Where a particle is described as
having a non-lamellar phase or form, this indicates that
at least the internal region of the particle should
adopt this form. Non-lamellar particles will generally
have two distinct regions, an internal region and a
surrounding surface region. The surface region, even in
a "non-lamellar" particle will often be lamellar or
crystalline and may be any phase ranging from a highly
ordered crystalline or liquid crystal phase to a
virtually orderless fluid layer. In contrast, a
"lamellar" particle, as described herein is a particle
having a solvent, rather than non-lamellar, core-region.

A "micellar" particle is used herein to indicate a
particle having no core region (in the sense that the
"core" is formed largely by the amphiphile molecules

themselves) such as in an L_2 phase, or is a
"microemulsion" particle in that it contains a core of a
solvent largely immiscible with the surrounding
continuous phase (e.g. an oil or oily amphiphile).
Preferred micellar dispersions are L_2 phase dispersions.

The term "lamellar particles" is used herein to indicate
vesicular particles characterised in that they comprise
one or more outer lamellar bilayers of amphiphile,
surrounding an inner solvent compartment where this
inner solvent is largely miscible with the surrounding
continuous phase.

The presence of particles of a particular phase
structure (e.g. non-lamellar form) will preferably be
assessed from a set of cryo-transmission electron
microscopy particle images. Such images will typically
show at least 30 particles, preferably they will show a
sample of more than 50 and most preferably more than 100
particles. The presence of non-lamellar particles may
also be assessed by X-ray scattering experiments.

The invention and the loading method of the invention
provide particles with a higher loading level than is
obtainable by equilibration at 37°C. Such particles
have a loading of at least 130% of the level achievable
by equilibration and generally between 150% and 700% of
that value. Preferably the loading should be at least
200% of the level achievable by equilibration and more
preferably at least 250%.

In the context of active agent loading, the terms
"equilibration" or "equilibration at 37°C" as used
herein may be taken to indicate a method comprising the
following steps:

I)   preparing a 1% (by weight) dispersion of amphiphile
particles in an aqueous solvent (e.g. water or 0.9%
NaCl);

II)   adding excess active agent to the dispersion (e.g.
15 mg to a total volume of 3 ml to give a saturated
solution)

III) incubating the sample at 37°C for 3 days,
optionally with gentle magnetic stirring (up to 300 rpm)
or on a rotating table (up to 1 rpm);

IV)   separating excess active agent from the dispersion
(e.g. by filtration through a 5 µm filter);

V)   analysing the active present in the dispersion
(e.g. by optional dissolution in 10 volumes of
water/acetonitrile/methanol (50/45/5) and analysis by
high performance liquid chromatography); and

VI)   calculating the percentage by weight of active
agent incorporated relative to the total weight of
amphiphile(s).

The skilled worker will have little difficulty in
reproducing this method but reference may also be made
to the Examples below for further details.

The period of 3 days has been chosen herein as a
suitable period for providing essentially maximum
loading of active agent by the equilibration method.

The loading of active agents has been found to plateau
at around 1 day as is, for example, indicated in Figure
1 attached hereto. This loading study can easily be
repeated by a skilled worker for any active/particle

combination.   Should it thereby be discovered that 3
days is insufficient to approach maximum loading in any
particular and case, a correspondingly increase the
equilibration time to 5, or even 10 or more days could
be made as appropriate.

As a comparison, particles of or loaded by the invention
may be compared with particles loaded by equilibration
by preparing particles of the invention and subjecting
them (after cooling to 37°C or preferably to room
temperature and preferably after equilibration at that
temperature for at least one hour) to steps IV to VI as
described above. The resulting percentage loadings can
then be expressed as a proportion, percentage or
percentage increase, relative to the loading provided by
the equilibration method.   As used herein, the term
"carrier capacity" is applied to the percentage weight
of incorporated active agent relative to that of total
amphiphile.

The temperature to which the particles must be heated in
order to provide the effect of the present invention
will be readily established by one of skill in the art.
For example, a sample of particles dispersed in a
saturated solution of active agent may be heated to a
particular temperature for 4 hours and subsequently
cooled (e.g. to ambient temperature). The loading level
of active agent (carrier capacity) may then be analysed
and compared to the loading provided by equilibration.
If no, or insufficient, increase is observed then the
experiment may be repeated at increased temperature.
Similarly, the length of time required for loading at
any particular temperature may be assessed by heating
suitable samples for set times and examining any changes
in loading level in comparison with an equilibrated
standard.   Equivalent heating experiments will also

determine the effect upon particle size distribution and
storage stability, using analytical tools such as high
performance liquid chromatography, light scattering and
cryo transmission electron microscopy.

Purely as a guide, samples will typically be heated to a
temperature in the range 75 to 200°C, preferably 85 to
150°C, more preferably 96 to 140°C. The most preferred
temperature range is 100 to 130°C. The heat may be
supplied by any appropriate method, such as by
autoclaving, baking in an oven, by electromagnetic
irradiation (e.g. infra-red or microwave irradiation)
and/or alternatives known in the art. Generally, where
heating is to a temperature in excess of around 100°C,
the dispersion will be sealed and/or pressurised to
avoid excessive loss of solvent.

Typical periods of heating at an elevated temperature
are relatively short and will generally be between 1
minute and 4 hours, more typically between 2 minutes and
1 hour. Periods of between 2 and 30 minutes are
preferred, particularly between 5 and 20 minutes. The
period may optionally include a period for temperature
equilibration, typically 1-10 minutes. Longer periods
may be used but, where no further or desirable benefit
to the carrier capacity is found by longer heating, this
will not typically be desirable.

The concentration of amphiphile in the dispersion of
amphiphile particles treated by the method of the
present invention is typically 0.1 to 20% by weight of
dispersion. More preferably this will be 0.3 to 10% and
most preferably in the range 0.5 to 7% (e.g. 1 to 5%).
The exact concentration used during the heat treatment
step will vary depending upon the desired final use of
the loaded particles and also upon the desired particle

size distribution. Heat treatment loading of the
invention can provide some simultaneous control over
particle size as indicated herein below.

The components of the amphiphile particles referred to
herein include at least one structuring agent (an
amphiphile) and will generally also include a
fragmentation agent (which may also be an amphiphile,
such as a surfactant, copolymer and/or protein,
preferably a surfactant). In addition, the invention
provides particles incorporating an active agent, which
includes protein, drug, nutrient, cosmetic, diagnostic,
pharmaceutical, vitamin, or dietary agents. Under some
circumstances the structuring agent or fragmentation
agent may also be an "active agent", in the sense of
being bioactive but, as used herein, the term "active
agent" does not include structuring agents,
fragmentation agents or solvents forming part of the
bulk composition. In one embodiment of the invention,
the active agent is not a structure-forming amphiphile
and is not a fragmentation agent, as described herein.
In a further embodiment, the active agent is not an
amphiphile.

The term structuring agents, as used herein, indicates
amphiphilic agents that are capable of forming (as the
major component - i.e. at greater than 50% by weight)
lamellar, micellar and/or non-lamellar phase structures,
optionally in the presence of other agents such as
fragmentation agents. Structuring agents will be
amphiphiles, having at least one polar, hydrophilic
group and at least one non-polar, hydrophobic group. A
wide range of structuring agents are applicable for use
as all or part of the structuring agent component.

Examples of polar groups are well known (see e.g. US

published patent application number 20020153509) and
include anionic groups such as carboxylates,
phosphonates, sulphates and sulphonates, non-ionic
groups such as alcohols, polyols (eg sugars, glycerol
etc) and esters, cationic groups such as quaternary
ammonium compounds, pyridinium salts and quaternary
phosphonium salts and zwitterionic groups such as
phospholipid head groups (e.g phosphatidyl-choline,
phosphatidic acid, phosphatidyl-ethanolamine,
phosphoglycerol, phosphoserine, their PEGylated or
mPEGylated derivatives, etc.), ammonioacetates, ammonio-
alkanesulphonates and trialkylaminoalkylphosphate
esters.

Examples of non-polar groups include C_6-C_{32} alkyl and
alkenyl groups, which are typically present as the
esters of long chain carboxylic acids. These are often
described by reference to the number of carbon atoms and
the number of unsaturations in the carbon chain. Thus,
CX:Y indicates a hydrocarbon chain having X carbon atoms
and Y unsaturations. Examples particularly include
caproyl (C6:0), capryloyl (C8:0), capryl (C10:0),
lauroyl (C12:0), myristoyl (C14:0), palmitoyl (C16:0),
phytanoyl (C16:0), palmitoleoyl (C16:1), stearoyl
(C18:0), oleoyl (C18:1), elaidoyl (C18:1), linoleoyl
(C18:2), linolenoyl (C18:3), arachidonoyl (C20:4),
behenoyl (C22:0) and lignoceroyl (C24:9) groups. An
amphiphile will typically have one or two non-polar
"tail" groups (mono-acyl and di-acyl lipids
respectively) but may have three, four or more
hydrophobic groups.

Examples of structuring agents suitable for use in the
present invention include natural lipids, synthetic
lipids, surfactants, and copolymers. Preferred agents
are glycerides (e.g. monoglycerides, diglycerides, and

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triglycerides), di- and polyglycerolesters of glycerides
(e.g. diglycerol monooleate, diglycerol monocaprate),
natural fats and oils (e.g. soybean oil, coconut oil,
corn oil, castor oil, sunflower oil), fractionated oils
(e.g. fractionated coconut oil, Miglyol® (Condea)),
transesterified oils (e.g. Maizine®),
transesterification products of oils and PEG (e.g.
ethoxylated castor oil (e.g. Cremophor® EL (BASF)),
ethoxylated hydrogenated castor oil (e.g. Cremophor®
RH-40 (BASF)), ethoxylated corn oil (e.g. Labrafil® M
2125 CS (Gattefossé))), acetylated monoglycerides, fatty
acids (e.g. C6-C26 saturated and unsaturated fatty
acids), fatty alcohols (e.g. phytantriol (3,7,11,15-
tetramethyl-1,2,3-hexadecantriol)), ether lipids (e.g.
monooleyl glyceryl ether), natural and synthetic
phospholipids (e.g. egg lecithin, soya lecithin,
hydrogenated lecithin, phosphatidyl choline,
phosphatidyl ethanolamine, phosphatidyl serine,
phosphatidyl glycerol, phosphatidic acid),
lysophospholipids (e.g. lyso-lecithin, lyso-phosphatidyl
choline, lyso-oleyl phosphatidyl choline),
phospholipid-analogous compounds (e.g. those disclosed
in US 6344576), sterols and sterol derivatives (e.g.
cholesterol, sitosterol, lanesterol and their esters,
especially with PEG or fatty acids), galactolipids (e.g.
digalactosyl diacylglycerol, monogalactosyl
diacylglycerol), sphingolipids (e.g. sphingomyelin);
nonionic surfactants, in particular ethoxylated
surfactants such as PEG-fatty acid mono- and diesters
(e.g. of the Crodet® (Croda), Cithrol® (Croda), Nikkol®
(Nikko), Myrj® (ICI) series, Solutol® HS 15 (BASF)), PEG
glycerol fatty acid esters (e.g. Tagat® L and O
(Goldschmidt), Glycerox® L series (Croda), Capmul® EMG
(Abitec)), transesterification products of oils and PEG
(e.g. of the Labrafil® (Gattefossé), Cremophor® (BASF)
Crovol® (Croda) and Nikkol® HCO (Nikko) series) ,

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PEG-sorbitan fatty acid esters (e.g. Tween® 20, Tween®
80 and other polysorbates of the Tween® series (ICI)),
PEG alkyl esters (e.g. of the Brij® (ICI) and Volpo®
(Croda) series), PEG alkyl phenol surfactants (e.g. of
the Triton X and N series (Rohm & Haas); polyglycerised
fatty acids (e.g. Nikkol® Decaglyn (Nikko), Plurol®
Oleique (Gattefossé)), propylene glycol fatty acid
esters), propylene glycol fatty acid esters (e.g.
Capryol® 90 (Gattefossé), Lutrol® OP2000 (BASF), Captex®
(Abitec)), glycerol/propylene glycol fatty acid esters
(e.g. Arlacel® 186 (ICI)), sorbitan fatty acid esters
(e.g. of the Span® (ICI) and Crill® (Croda) series),
sugar esters (e.g. of the SUCRO ESTER® (Gattefossé) and
Crodesta® (Croda) series),
polyoxyethylene-polyoxypropylene block copolymers
(so-called poloxamers, e.g. of the Pluronic® (BASF),
Synperonic® (ICI) and Lutrol® (BASF) series), copolymers
of ethylene oxide and butylene oxide; anionic
surfactants including fatty acid salts, bile salts (e.g.
sodium cholate, sodium glycocholate, sodium
taurocholate), carboxylates such as ether carboxylates,
succinylated monoglycerides, mono/diacetylated tartaric
acid esters of mono- and diglycerides, citric acid
esters of mono- and diglycerides, glyceryl-lacto esters
of fatty acids, acyl lactylates, alginate salts,
propylene glycol alginate; cationic surfactants
including ethoxylated amines (e.g. polyoxyethylene-15
coconut amine), betaines (e.g.
N-lauryl-N,N-dimethylglycine), alkylpyridinium salts,
quarternary ammonium salts such as hexadecyl triammonium
bromide, decyl trimethyl ammonium bromide, cetyl
trimethyl ammonium bromide; zwitterionic surfactants
including trimethylammonioethylalkylphosphonates (e.g.
the examples disclosed in US 6344576); and all mixtures
thereof. The most preferred structuring agents are
glycerol and diglycerol monooleate and monolinoleate,

glyceoldioleate (GDO), dioleyl phosphatidyl ethanolamine
(DOPE), dioleyl phosphatidylcholine (DOPC) and
phytantriol, and mixtures of these with up to 50% fatty
acids, in particular oleic acid and linoleic acid,
polysorbate 80 (Tween® 80), polyethylene glycol 660
12-hydroxysterate (Solutol® HS 15), or
lyso-phospholipids, especially lyso-oleyl
phosphatidylcholine (LOPC).

Often the structure forming agent component will contain
components in the form of extracted and purified natural
products and will thus contain a mixture of related
compounds. Soy bean phosphatidyl choline, for example
is a mixture of compounds having around 60-75% C18:2
acyl groups, around 12-16% C16:0 and the balance others.
Similarly, commercial glycerol monooleate is typically
at least 90% monoglyceride but contains small amounts of
diglyceride and free fatty acid, with the acyl groups
being over 60-90% C18:1, 5-10% saturated and the
remainder largely higher unsaturated acyl groups.

A highly preferred structuring agent for use in the
present invention is commercially available glycerol
monooleate (GMO). As indicated above, this is largely
monoglyceride with an oleoyl (C18:1) acyl chain but
contains certain amounts of other compounds. These are
included in the term "glycerol monooleate" or "GMO" as
used herein. Commercial preparations of GMO include
GMOrphic-80 and Myverol 18-99 (available from Eastman
Kodak), Rylo MG 19 and Dimodan DGMO (available from
Danisco). Any of the structuring agents may be used
alone or in combination with one or more other
structuring agents. Other preferred structuring agents
include Diglycerolmonoacyl lipids such as
Diglycerolmonooleate (DGMO) and glyceroldiacyl lipids
such as glyceroldioleate (GDO), as well as mixtures

thereof.

As all, or preferably a portion of, the amphiphilic
structuring agent component, the particles of the
invention may, in particular, include at least one fatty
acid or fatty acid salt component. Preferred fatty
acids have between 6 and 24 carbons and particularly
those corresponding to the fatty acid chains of natural
lipids, including caproic, caprylic, capric, lauric,
myristic, palmitic, phytanic, palmitolic, stearic,
oleic, elaidic, linoleic, linolenic, arachidonic,
behenic or lignoceric acids, their salts or mixtures
thereof. The fatty acids may be saturated but are
preferably unsaturated. The most preferred fatty acid
is oleic acid. Salts of fatty acids will typically be
physiologically tolerable, and for pharmaceutical
applications will always be so. Preferred salts include
alkali and alkaline earth metal salts such as sodium,
potassium, lithium, calcium or magnesium salts as well
as ammonium and alkylammonium salts. Typically, the
fatty acid or fatty acid salt will be present as 0-10
wt% of the total amphiphilic component, preferably 3-7%
by weight.

Particularly where the method of the present invention
is used to load microemulsion "micellar" dispersions, at
least a portion of the structuring agent component may
be one or more oily components being largely immiscible
with water. Typical oily amphiphiles have a Hydrophilic-
Lipophilic Balance (HLB) of 4 or less. Preferred
examples of such oily structuring agents include
triglycerides, diesters of ethylene or propylene glycol,
fatty acid / fatty alcohol esters and tocopherols.
Where these low HLB amphiphiles are used, it will
usually be necessary to include at least one surfactant
type fragmentation agent, especially where the portion

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of oily amphiphile is high.

The fragmentation agents for use in the particles
referred to herein will be at least one agent which aids
the dispersal of the structuring agent particles
(preferably micellar or particularly non-lamellar
particles) or stabilises such particles. Typically a
fragmentation agent will be a surfactant such as an
amphiphilic block copolymer. A large number of
surfactants and copolymers are suitable for use as all
or part of the fragmentation agent for use in the
present invention. Typically fragmentation agents have
an HLB of at least 12, preferably at least 14.

Important fragmentation agents include natural lipids,
synthetic lipids, surfactants, copolymers, proteins (in
particular caseins and albumin), hydrotropes, alcohols
and other additives that may facilitate fragmentation
spontaneously or with the aid of externally applied
forces and pressures and contribute to stabilisation.
This includes also nanoparticles and combinations of
polymer and nanoparticles (see e.g. WO 99/12640).

Suitable copolymers for use as fragmentation agents may
have blocks comprising polyoxyalkylenes,
polyvinylpyrollidone, polyvinylacetate,
polyvinylalcohol, polyesters, polyamides and/or
polyalkenes. The block copolymer will comprise at least
two blocks of polymer having different degrees of
hydrophilicity.

Certain proteins (such as casein) are also of
amphiphilic character and may be used as fragmentation
agents but are not preferred.

Preferred examples of amphiphilic block copolymers are

poloxamers, which comprise at least one block of
polyoxyethylene and at least one block of
polyoxypropylene. The most preferred fragmentation
agents are poloxamer 407 (e.g. Pluronic® F127, BASF),
poloxamer 188 (e.g. Pluronic® F68, BASF), poloxamer 124
(Pluronic® L44, BASF), and polysorbates 20, 60 and/or 80
(referred to herein a P20, P60 & P80 respectively - e.g.
Tween® 80, ICI). Other suitable surfactants copolymers
may be found in the "Handbook of Pharmaceutical
Excipients" (2nd Ed., the American Pharmaceutical
Association and The Pharmaceutical Press, Royal
Pharmaceutical Society of Great Britain).

Other preferred fragmentation agents include
polyethylene glycol lipid conjugates (e.g. PEGylated and
mPEGylated phospholipids) as well as long chain alcohols
and fatty acids.

Particularly preferred fragmentation agents include
PEGylated castor oils (Cremophors), polysorbates (P80),
any PEGylated lipids with more than about 20 PEG units
per molecule, block copolymers (e.g. PEG-PPG block
copolymers - poloxamers), hydrophobically modified (hf)
polymers (hf polysaccharides such as hf starch, hf
polyacrylates and hf cellulose derivatives),
polyglycerin attached phospholipids (e.g. Coatsome EL
Series from NOF), cholesterol pullulan (NOF) and
2-Methacryloyloxyethyl phosphorylcholine n-butyl
methacrylate co-block polymers (PUREBRIGHT MB-37-50T and
PUREBRIGHT MB-37-100T from NOF).

Since the amphiphiles for use in the method of the
invention or comprised in the particles of the invention
are subjected to heat treatment, it is desirable that
they be stable to these conditions. Typical structuring

agents will generally be sufficiently stable to avoid
degradation under these conditions but certain possible
fragmentation agents, such as peptides, are more
temperature sensitive and are thus not preferred. All
components of the particles should be stable to the
conditions of heat treatment and this can be tested as
described herein.

In a preferred embodiment of the present invention, the
particles referred to herein have amphiphilic components
comprising at least one structure forming amphiphile
(component a), at least one "structure swelling" agent
(component b) and at least one dispersion stabilising
"polymeric" agent (component c). Components b and c
will also act as fragmentation agents. In this
embodiment, at least 50% by weight of the total
amphiphilic components (a+b+c) should be component a.
Preferably this will be 60 to 95%, more preferably 70 to
90%. Correspondingly, component b should be less than
40% by weight of a+b+c, preferably 5 to 30% and more
preferably 10 to 25%. Component c should be present at
less than 20%, preferably 1 to 15% and more preferably 2
to 10% of the total weight of a+b+c.

Compositions comprising components a, b and c as
described herein ("ternary compositions") are highly
suitable for use in the methods of the present invention
and in particular are highly suitable for forming
desirable non-lamellar particles for loading by the
method of the invention. Such particles typically have
a thermodynamically stable non-lamellar state at room
temperature in an appropriate aqueous medium.
Furthermore, the compositions may have favourable *in
vivo* properties such as low haemolytic effects and low
acute toxicity, thereby providing enhanced utility as
carriers for active agents such as drugs and/or

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nutrients (see active agents indicated herein).

In the ternary amphiphilic compositions, structure
forming component "a" will preferably comprise at least
one lipid component such as glycolipids, diglycerides
and/or phospholipids (e.g phosphatidyl ethanolamines).
Naturally occurring lipids are particularly suitable but
non-naturally occurring variants such as ether lipids
(having a head and tail group joined by an ether bond)
are also suitable. Lipids such as diacyl phosphatidyl
ethanolamines, and diacylglycerols and diacyl
phosphatidyl cholines are highly suitable.

In this embodiment, component a may also contain up to
10% (e.g. 1-10% by weight of this component) of at least
one charged amphiphile, particularly anionic lipids
(such as acyl or diacyl phosphatidyl glycerols) or fatty
acid (see above). Correspondingly, 90% or more,
preferably at least 95% of the component a should
preferably have no net charge under neutral and/or
physiological conditions. Component a when formulated
alone in excess water should form a reversed non-
lamellar phase, preferably a reversed hexagonal phase.

The structure swelling component "b" is generally a
component which swells the lattice of the amphiphilic
structure allowing it to more readily be dispersed into
particulate form. This component may also facilitate
structural transition, for example, from reversed cubic
to hexagonal phase structures. Structure swelling
agents will generally have a relatively low molecular
weight (e.g. less than 2000) and are preferably
components such oligoethylene oxide based surfactants.
Preferred examples oligoethylene oxide based surfactants
are those having between 5 and 40 ethylene oxide units
bonded to a non-polar "tail" group (e.g. as an ester to

a fatty acid, such as any of those described herein, or
as an ether to a corresponding fatty alcohol).
Preferred examples include polyoxyethylene alkylethers,
polyoxyethylene sorbitan fatty acid esters
(polysorbates), polyoxyethylene sterates,
polyoxyethylene castor oil derivatives and
polyoxyethylene lipid derivatives. Most preferred
examples are TMGO-15 (Nikko), Solutol HS15 (BASF) and
polysorbate 80.

The polymeric component "c" is, in general, a component
which improves the stability of the dispersion,
particularly as colloidal particles. Polymeric
components generally have a relatively high molecular
weight (e.g. greater than 2000) and will have at least
one polymeric or copolymeric portion in their molecular
structure. Preferred polymeric components include
polyethylene oxide copolymers and lipids derivatised
with polyethylene oxide, hydrophobically modified
polysaccarides and amphiphilic proteins. Poloxamers as
described herein are particularly suitable as the
polymeric components as are PEG-substituted lipids such
as PEG-glyceroldioleate, PEG-dioleoyl phosphatidyl
ethanolamine (in particular DOPE-PEG2000 and DOPE PEG-
5000) or PEG-dioleoyl phosphatidyl serine.

Preferred examples of polyethylene oxide copolymers are
poloxamers, which comprise at least one block of
polyoxyethylene and at least one block of
polyoxypropylene. The most preferred of these agents
are poloxamer 407 (e.g. Pluronic® F127, BASF), poloxamer
188 (e.g. Pluronic® F68, BASF), poloxamer 124 (Pluronic®
L44, BASF).

In all particles referred to herein, fragmentation
agent(s) will be present at a level sufficient to bring

about the fragmentation of the structuring agent and/or
to stabilise the particles. In some cases no
fragmentation agent will be required but in others,
particularly in order to disperse non-lamellar phases,
fragmentation agent(s) will be necessary. The particles
may form spontaneously or may require physical
fragmentation such as by shearing and/or
ultrasonication. It is preferable that sufficient
fragmentation agent is present that the particles are
physically stable.

Preferred combinations of structure forming agents and
fragmentation agents include combinations of GMO, GDO,
DGMO and/or DOPE with at least one of Poloxamer 407,
Poloxamer 188, TMGO-15/DOPE-PEG(5000) and/or P80.

In one preferred embodiment, the particles of and for
loading in the method of the present invention consist
of GMO and one or more fragmentation agents (such as
poloxamers), with an active agent after loading and
optionally with an aqueous component.

Active agents suitable for inclusion in the methods and
formulations of the present invention include human and
veterinary drugs and vaccines, diagnostic agents,
"alternative" active agents such as plant essential
oils, extracts or aromas, cosmetic agents, nutrients,
dietary supplements etc. The active agents will
desirably be stable to the heat treatment loading method
and the particular amphiphilic particles, temperature
and heating time chosen for use in the loading method
will take into account active agent stability. When
suitable conditions are chosen, the active agent(s)
should be stable as indicated herein above. Virtually
any active agent satisfying this requirement may be used
in the method and particles of the invention.

It is preferred, in order to provide the greatest
increase in loading, that the active agent have a
relatively low aqueous solubility. Preferably, the
active agent will have a high logP (being the log to
base 10 of the partition coefficient of the active agent
between water and n-octanol), such as logP > 2,
preferably logP > 2.5 and more preferably logP > 3.
Preferably the active agents will have a relatively low
melting point, such as below 170°C, preferably below
150°C and most preferably between 100°C and 140°C.

Examples of suitable drugs include steroids, sparingly
soluble weakly basic drugs, fibrins, statins, dipins,
and azoles. Specific preferred examples of these
include progesterone, testosterone, simvastatin,
lovastatin, nifedipin, felodipin, nicardipin, nimodipin,
itraconazole, fluconazole, miconazole, econazole,
voriconazole, clotrimazole, ketoconazole, fulvestrant,
fenofibrate, octreotide, undecanoate, estradiol,
cortisone, hydrocortisone, 11a-hydroxyprogesterone,
clofibrate gemfibrozil, bezafibrate, ciprofibrate. Some
categories of active agents, such as peptides, are less
preferred due to their limited heat stability in aqueous
solution. Certain peptides, especially cationic and
cyclic peptides, however, are more temperature stable
and these are suitable for use in the invention.
Octreotide is an example of a temperature stable
peptide. Most preferred active agents are progesterone,
ketoconazole, fulvestrant, fenofibrate, octreotide and
testosterone.

Diagnostic agents include radionuclide labelled
compounds and contrast agents including X-ray,
ultrasound and MRI contrast enhancing agents. Nutrients
include vitamins, coenzymes, dietary supplements etc.
The active agents for use in the present invention will

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generally not be poloxamers or acylglycerols.

An active agent will frequently have an effect upon the
phase behaviour of the structuring agent(s). For
example, certain active agents (such as cyclosporin A)
introduce greater negative curvature than some
structuring agents and at high concentrations may cause
the formation of highly negatively curved phases, such
as the reversed micellar L_2 phase rather than a cubic or
hexagonal liquid crystalline phase. Nonetheless, such
an active agent could be formulated into, for example, a
reversed hexagonal phase by formulation with a
structuring agent, or a blend thereof, having a less
negative spontaneous curvature. By this method, the
overall mixture provides the appropriate negative
curvature to allow use in the methods or compositions of
the invention.

The skilled worker will be able to use standard methods
to assess the degree of spontaneous curvature of any
particular structuring agent (or mixture thereof) or the
effect on this by including an active agent. This might
be done, for example, by studies of the bulk phase
behaviour of each structuring agent in water and
subsequent studies with varying concentrations of active
agent included. The phases can be examined by any of
the methods indicated herein (e.g. polarised light, SAXS
cryo-TEM etc.) and an appropriate blend of structuring
agents chosen for each case. In some circumstances,
where the effect of the active agent on the phase
behaviour of the mixture is significant, the structuring
agent(s) chosen may not provide the desired phase in
themselves (e.g. may have too small or too great
spontaneous curvature) but will generate this phase only
when also formulated with the active agent. Similarly,
the equilibrium phase may change from, for example,

cubic to hexagonal liquid crystalline phase upon
addition of the active agent.

The loading method of the present invention may also be
used to simultaneously load active agent into
amphiphilic particles and to control the particle size
and size distribution. The inventors have established
that average particle size is generally increased by
heating in media with higher ionic strength. Typically,
stable, (especially non-lamellar) particle dispersions
may be formed by carrying out the heat treatment loading
step at ionic strength in the range 0.1 mM to 100 mM
NaCl (or ionic strength equivalent) depending upon the
composition used. The precise size distribution will
depend upon the composition and suitable conditions may
quickly be established by reference to the methods
described herein, but typically sub-micron particles are
formed at low ionic strength and larger colloidal and
non-colloidal particles at increasing ionic strengths.

Where small particles are required in solutions of
relatively high salt concentrations (e.g. in 0.9% NaCl
for injections) the particles may be formed by the heat
treatment method of the invention at a low ionic
strength and, after cooling, further salt(s) added to
provide the desired osmolality.

In a yet further embodiment of the invention, the
present inventors have further established that the
particle size distribution of a formulation comprising
at least one structuring agent may be further controlled
by carrying out the loading method of the present
invention in an aqueous medium at controlled
concentration of amphiphile. In particular, small (e.g.
colloidal, especially small colloidal (<0.3 μm))
particles are most easily formed at low concentration of

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amphiphile, such as below or around 10 wt% total
amphiphile in aqueous solution

Where small particles are required at relatively high
concentrations of amphiphile (e.g. to minimize the total
volume for injections) the particles may be formed by
the methods of the invention at high dilution and, after
cooling, concentrated by evaporation, ultrafiltration
etc. Conversely, where larger particles are required at
high dilutions (e.g. for infusion to a subject) then
these may be formed by the processes described herein at
high concentrations and, once cooled, diluted further.

In the methods of the invention, particles comprising a
structuring agent are formed prior to one or more heat
treatment cycles. This pre-formulation will typically
be in the form of a dispersion and may be prepared by
established methods. Methods for forming lamellar (e.g.
liposomal) and micellar (including microemulsion)
dispersions are well known and several methods for
forming dispersions of non-lamellar phase particles are
also well established, such as those indicated in the
present Examples and in US 5,531,925, WO 02/02716,
WO 02/068561, WO 02/066014 and WO 02/068562. The
disclosures of these and all references cited herein are
hereby incorporated herein by reference.

Methods for forming dispersions of non-lamellar phase
particles include adding an amphiphile/water liquid
crystal phase to an aqueous solution of fragmentation
agent and optionally a lipid (such as PC) and either
allowing natural fragmentation of the mixture or
accelerating the process with, for example, mechanical
agitation, vortexing, roto-stator mixing, high-pressure
homogenization, microfluidisation and/or ultrasound.

After heat-cycling enhanced loading, the particles may
be concentrated (e.g. by ultrafiltration or dialysis)
and/or dried, for example by spray drying, fluid bed
drying or freeze drying. In the case of dried
particles, the drying process may be followed by
particle size enlargement through single or repeated
agglomeration and granulation steps. The concentrated,
dried and/or agglomerated particle formulations thus
formed may be used as such or hydrated and/or dispersed
to yield particle dispersions suitable for use in the
delivery of active substances, especially *in vivo*. Such
concentrated, dried and/or agglomerated particle
formulations and the dispersions resulting from their
re-suspension/hydration form further aspects of the
present invention. Drying may be carried out in the
presence of protective and/or resuspension promoting
agents such as hydrophilic polymers or sugars, as is
well known in the art.

In one embodiment of the invention, an initial
pre-formulation, prior to heat treatment loading, is
formed in which the particles will preferably be small
colloidal sized particles, for example in the range 0.02
to 0.2 μm. Preferably the mean particle size for the
small colloidal particles will be 0.05 to 0.15 μm in
this pre-formulation.

The loaded particles of the invention will preferably
have average (mean) sizes below 10 μm, more preferably
below 5μm and most preferably below 1 μm. In one
embodiment, after loading by one or more heating and
cooling cycles, the final particles should be in the
colloidal size range. These will typically have an
average (mode or preferably mean) particle size in the
range 0.05 to 1μm, preferably 0.1 to 0.8 μm (e.g. 0.2 to
0.8 μm), more preferably 0.2 to 0.6 μm (e.g. 0.3 to 0.6

μm). It is particularly important that preparations for use in intravenous administration should not contain particles in the non-colloidal range (e.g >1μm or particularly >5 μm, and especially >10 μm, as indicated herein). For intravenous applications a preferred particle size range is 0.05 to 0.3 μm. This may be achieved by using the method of the invention, beginning with small colloidal particles as described above. Alternatively, or in addition, the particles, preferably after heat cycle loading, may be filtered in order to remove larger (e.g. non-colloidal) particles. In colloidal formulations, preferably no more than 10% of particles will be outside the range 0.05 to 10 μm, more preferably, not more than 1% will be outside this range, and most preferably no detectable (by laser diffraction) proportion of particles will be outside this range.

The particles formed or formable by the method of the invention may be used in the production of nutritional, dietary, cosmetic, diagnostic veterinary or pharmaceutical compositions by known methods using well known carriers, excipients and other ingredients. In the case of pharmaceutical compositions, the particles will be formulated with at least one pharmaceutically acceptable carrier or excipient and may be formed into tablets, capsules and so forth. The particles may also be formulated as a pre-prepared dispersion in an acceptable liquid, such as water, or dried (e.g. spray dried or freeze dried) and sealed in sterile containers for re-suspension prior to administration.

The present inventors have surprisingly established that the loading of active agent into amphiphilic compositions may be enhanced by one or more cycles of heat treatment as describe herein. A method of loading an active agent by heat cycling thus forms an aspect of

the invention, as do the products formed thereby.

In this aspect of the invention, the active agent must
be stable to the conditions of the heat cycling. The
active agents should thus be chemically stable in
aqueous environments under the conditions of heat and
duration described herein. The suitability of any
active agent for this aspect of the invention may be
established by routine testing under the heat-cycling
conditions described herein. Preferred active agents in
this respect include steroids such as progesterone,
adrenocortical hormones, gonadal hormones, cardiac
aglycones, bile acids and sterols. Progesterone is
particularly preferred.

Upon heat treatment by the method of the present
invention in the presence of heat-tolerant active agents
it has been noted that a loading level of several times
that achieved by loading at room temperature can be
generated. That is, at least twice the quantity of
active agent can be incorporated into amphiphilic
compositions described herein by heat treatment than can
be incorporated by equilibration at room temperature.
This ratio can be 3, 4 or 5 times and may be up to 6 or
more with certain active agents. Furthermore, whether
or not the active agent solubilised by this method is in
a meta-stable state or in a truly stable dispersion or
solution, the compositions loaded with up to 6 times the
room temperature equilibrium level with active agent
(especially steroid) have been observed to be stable to
storage for at least two weeks. This offers
considerable and obvious advantages in being able to
provide high drug loads at while administering a small
volume and low level of carrier to the subject.

The invention will be illustrated below by the following
non-limiting examples and the accompanying figures in
which:

Figure 1 shows the loading of progesterone into
amphiphile particles by equilibration over 3 days.

Figure 2 shows the increase in loading (carrier
capacity) when progesterone (PRO) is loaded into each of
8 amphiphile dispersions by heating at 125°C for 20
minutes in comparison with gentle stirring for 3 days at
37°C;

Figure 3 shows the increase in loading (carrier
capacity) when fenofibrate (FFT) is loaded into each of
8 amphiphile dispersions by heating at 125°C for 20
minutes in comparison with gentle stirring for 3 days at
37°C;

Figure 4 shows the increase in loading (carrier
capacity) when fulvestrant (FUL) is loaded into each of
8 amphiphile dispersions by heating at 125°C for 20
minutes in comparison with gentle stirring for 3 days at
37°C;

Figure 5 shows the increase in loading (carrier
capacity) when ketoconazole (KET) is loaded into each of
8 amphiphile dispersions by heating at 125°C for 20
minutes in comparison with gentle stirring for 3 days at
37°C;

Figure 6 shows the increase in loading (carrier
capacity) when testosterone (TES) is loaded into each of
8 amphiphile dispersions by heating at 125°C for 20
minutes in comparison with gentle stirring for 3 days at
37°C;;

Figure 7 shows a comparison of the relative increases in loading (carrier capacity) for 5 active agents loaded into up to 8 amphiphile dispersions by heating at 125°C for 20 minutes in comparison with gentle stirring for 3 days at 37°C;

Figure 8 shows the effect upon particle size of storage at 25°C for 3 weeks for amphiphile dispersions with and without progesterone loaded to 10% carrier capacity;

Figure 9 shows the effect upon particle size of storage at 37°C for 3 weeks for amphiphile dispersions with and without progesterone loaded to 10% carrier capacity;

Examples:

The following materials were used in the Examples and are abbreviated as shown:

GMO   glyceryl monooleate (obtained from Danisco)

OA   oleic acid (Apoteket)

DGMO   diglyceryl monooleate (Danisco),

GDO   glyceryl dioleate (Danisco),

F127   Pluronic F127 (Sigma),

DOPE   dioleoyl phosphatidylethanolamine (Avanti),

TMGO-15 PEG-15 glyceryl monooleate (Nikko),

DGMC   diglyceryl monocaprinate (Danisco),

PC   soybean phosphatidylcholine,

         Epikuron 200 (Degussa),

         Maisine (Gattefossé),

CrRH40   Cremophor RH40 (BASF),

P80   Polysorbate 80 (Apoteket),

PG   propylene glycol (Apoteket),

EtOH   ethanol 99.5% (Kemetyl).

Example 1 - Preparation of loading compositions

Nine different aqueous loading systems were prepared at
an amphiphile concentration of 1% by weight (Table 1).

Table 1. Compositions of loading systems

System	GMO	OA	DGMO	GDO	F127	DOPE	TMGO-15	PEG5000-DOPE	DGMC	PC	Maisine	Cr RH40	P80	PG	EtOH	water
1	0.9	-	-	-	0.1	-	-	-	-	-	-	-		-	-	99
2	0.86	0.04	-	-	0.1	-	-	-	-	-	-	-		-	-	99
3	-	-	0.54	0.36	0.1	-	-	-	-	-	-	-		-	-	99
4	-	0.025	-	-	-	0.8	0.15	0.025	-	-	-	-		-	-	99
5	-	-	1	-	-	-	-	-	-	-	-	-		-	-	99
6	-	-	-	-	-	-	-	-	1	-	-	-		-	-	99
7	-	-	-	-	-	-	-	-	-	1	-	-		-	-	99
8	-	-	-	-	-	-	-	-	-	-	0.45	0.54	0.13	-	0.13	98.75
9	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	99

Predispersions of systems 1-3 were prepared by dropwise
adding molten amphiphile mixture to water after which
the dispersions were allowed to equilibrate for about 1
day. Predispersions of systems 4, 5, and 7 were prepared
by swelling the amphiphiles in water and "freeze-thaw"
cycling the mixture three times, including freezing at -
85°C and thawing under vigorous stirring and shaking at
ambient temperature. All predispersions were homogenized

in a microfluidizer at high pressure (350 bar) for 15
min at room temperature. The dispersions were then
autoclaved at 125°C for 20 minutes and were allowed to
cool to ambient temperature before further use. Final
dispersions of systems 6, 8, and 9 were prepared by
adding amphiphile (and cosolvent) to water and allowing
the dispersions to equilibrate for at least 6 hours.

Example 2 – Loading of progesterone

15 mg of progesterone (PRO, Sigma) was added to vials
each containing 3ml of dispersion systems 1, 2, 3, 4, 5,
7, 8 (see Table 1), respectively. Four samples of each
composition were prepared and water samples were
prepared as a reference. Two samples of each composition
were equilibrated at 37°C for three days by gentle
stirring on a rotating table. The remaining samples were
heat treated by autoclavation at 125°C for 20 minutes
and were allowed to temperature equilibrate at 37 °C for
at least one hour before further processing. Undissolved
progesterone was separated from the dispersions by
filtration through a 5 μm cutoff hydrophilic filter. The
dispersions were dissolved by diluting ten times with a
50/45/5 mixture of water/acetonitrile/methanol.

Analysis of progesterone was performed on a binary HPLC
pump equipped with an autosampler, a degasser and a
variable-wavelength UV-VIS detector. A HiChrom ACE-5 CN
column (50 * 4.6 mm) with 5 μm particles was used at
ambient temperature.

Progesterone was eluted using a binary gradient from
solvent A: 0.02 % H_3PO_4 in water and B: acetonitrile. The
gradient composition was: time$_0$ (t$_0$) = 25 % B, t$_4$ = 65 %
B, t$_5$ = 25 % B and t$_6$ = 25 % B. The flow rate was 1.5
mL/min and UV detection was performed at 254 nm.

100 μL of a sample was diluted with 900 μL of sample
solvent, water/acetonitrile/methanol (50:45:5), for
samples of low concentrations, and further diluted 10
times for high concentration samples. A 20 μL aliquot
was injected on the HPLC.

Quantification of progesterone peaks was performed by
comparing the peak area of a sample with a linear
regression of calibration samples with known
concentrations (0.025 - 200 μg/mL) mixed in sample
solvent.

A loading time study was performed for the constant
temperature (37 °C) loading procedure and it was
concluded that plateau loading was achieved after about
1 day (Figure 1).

The resulting loading data were presented as "carrier
capacity" (Figure 2, Table 2), which is defined as the
weight percentage loaded progesterone per amphiphile
(i.e. excluding the solvent).

Table 2. Loading of progesterone

System	Carrier capacity / % Stirring 3 days 37°C	Stdev	Carrier capacity / % Autoclavation 20 min 125°C	Stdev	Relative increase	Increase (%)
1-PRO	3.32	0.02	15.96	1.34	4.8	381
2-PRO	2.90	0.02	18.45	0.86	6.4	537
3-PRO	2.99	0.01	12.65	1.65	4.2	323
4-PRO	1.51	0.02	9.34	2.82	6.2	520
5-PRO	3.21	0.05	19.64	0.01	6.1	512
7-PRO	2.43	0.03	4.10	0.21	1.7	69
8-PRO	3.39	0.11	17.61	3.71	5.2	419
Ref-PRO	0.09	0.00	0.60	0.02		

where stdev is the standard deviation, and increase and relative increase refer to the increase
in carrier capacity by heat treatment over constant temperature stirring.

Example 3 - Loading of fenofibrate

15 mg of fenofibrate (FFT, Sigma) was added to vials containing each 3ml of dispersion systems 1-8 (see Table 1), respectively. The samples were treated in the same way as described in Example 2.

Analysis of fenofibrate was performed similar to described in Example 2 with the following exceptions: A HiChrom ACE-5 C18 column (50 * 4.6 mm) with 5 μm particles was used at ambient temperature. Fenofibrate was eluted in isocratic mode using a mobile phase consisting of 0.02 % H_3PO_4 in water - acetonitrile (20:80, v/v). The flow rate was 1.5 mL/min and UV detection was performed at 300 nm.

The resulting carrier capacities are presented in Figure 3 and Table 3.

Table 3. Loading of fenofibrate

System	Carrier capacity / % Stirring 3 days 37°C	stdev	Carrier capacity / % Autoclavation 20 min 125°C	stdev	Relative increase	Increase (%)
1-FFT	2.9	0.09	8.41	1.32	2.9	188
2-FFT	2.8	0.03	9.47	1.05	3.4	244
3-FFT	3.3	0.08	7.12	0.04	2.2	118
4-FFT	1.7	0.23	5.16	0.46	3.1	207
5-FFT	2.7	0.04	4.94	0.66	1.8	81
6-FFT	2.4	0.04	2.74	0.02	1.2	16
7-FFT	1.8	0.01	2.19	0.08	1.2	21
8-FFT	6.0	0.28	10.88	0.99	1.8	82
Ref-FFT	0.0	0.00	0.02	0.00		

where stdev is the standard deviation, and increase and relative increase refer to the increase in carrier capacity by heat treatment over constant temperature stirring.

Example 4 - Loading of fulvestrant

15 mg of fulvestrant (FUL) was added to vials containing each 3ml of dispersion systems 1-5 and 7-9 (see Table 1), respectively. The samples were treated in the same way as described in Example 2.

Analysis of fulvestrant was performed similar to described in Example 2 with the following exceptions: A HiChrom ACE-5, C18, 5 μm, column (50 * 3,0 mm) with precolumn (10 * 3,0 mm) was used at ambient temperature. Fulvestrant was eluted using a binary gradient from solvent A: 0.02 % H_3PO_4 in water and solvent B: acetonitrile. The gradient composition was: time$_0$ (t_0) = 50 % B, t_3 = 80 % B, t_4 = 80 % B, $t_{4,1}$ = 50 % B and t_7 = 50 % B. The flow rate was 0,7 mL/min and UV detection was performed at 280 nm.

The resulting carrier capacities are presented in Figure 4 and Table 4.

Table 4. Loading of fulvestrant

System	Carrier capacity / % Stirring 3 days 37°C	stdev	Carrier capacity / % Autoclavation 20 min 125°C	stdev	Relative increase	Increase (%)
1-FUL	0.62	0.02	1.90	0.04	3.1	208
2-FUL	0.49	0.03	2.29	0.20	4.7	368
3-FUL	0.60	0.03	3.76	0.48	6.3	527
4-FUL	0.25	0.01	1.60	0.25	6.4	541
5-FUL	0.69	0.04	1.34	0.16	1.9	94
7-FUL	0.62	0.02	1.77	0.09	2.8	185
8-FUL	1.66	0.03	3.14	0.32	1.9	89
9-FUL	0.85	0.03	3.19	0.12	3.8	277
Ref-FUL	0.00	0.00	0.02	0.00		

where stdev is the standard deviation, and increase and relative increase refer to the increase in carrier capacity by heat treatment over constant temperature stirring.

Example 5 - Loading of ketoconazole

15 mg of ketoconazole (KET, Recordati) was added to vials containing each 3ml of dispersion systems 1-8 (see Table 1), respectively. The samples were treated in the same way as described in Example 2.

Analysis of ketoconazole was performed similar to described in Example 2 with the following exceptions: A HiChrom ACE-3 C18 column (100 * 3.0 mm) with 3 μm particles was used at 45°C. Ketoconazole was eluted using a binary gradient from solvent A: tetrabytulammonium hydrogensulfate, 3.4 g/L in water and B: acetonitrile. The gradient composition was: time 0 (t_0) = 10 % B, t_{14} = 65 % B, t_{16} = 65 % B, t_{18} = 10 % B and t_{24} = 10 % B. The flow rate was 0.65 mL/min and UV detection was performed at 225 nm.

The resulting carrier capacities are presented in Figure 5 and Table 5.

Table 5. Loading of ketoconazole

System	Carrier capacity / % Stirring 3 days 37°C	stdev	Carrier capacity / % Autoclavation 20 min 125°C	stdev	Relative increase	Increase (%)
1-KET	5.21	0.18	19.16	1.46	3.7	268
2-KET	4.41	0.01	14.01	0.06	3.2	218
3-KET	3.49	0.10	19.25	0.28	5.5	452
4-KET	2.40	0.14	5.82	0.30	2.4	143
5-KET	4.43	0.10	19.85	2.49	4.5	348
6-KET	3.32	0.01	5.82	0.42	1.8	75
7-KET	2.72	0.02	4.96	0.22	1.8	83
8-KET	2.50	0.01	18.07	0.81	7.2	624
Ref-KET	0.02	0.00	0.69	0.01		

where stdev is the standard deviation, and increase and relative increase refer to the increase in carrier capacity by heat treatment over constant temperature stirring.

Example 6 - Loading of testosterone

15 mg of testosterone (TES, Fluka) was added to vials containing each 3ml·of dispersion systems 1-2, 4-7 and 9 (see Table 1), respectively. The samples were treated in the same way as described in Example 2.

Analysis of testosterone was performed similar to described in Example 2 with the following exceptions: A HiChrom ACE-5, C18, 5 μm, column (50 * 3,0 mm) with precolumn (10 * 3,0 mm) was used at ambient temperature. Testosterone was eluted using a binary gradient from solvent A: 0.02 % H_3PO_4 in water and solvent B: acetonitrile. The gradient composition was: time$_0$ (t_0) = 40 % B, t_2 = 70 % B, t_3 = 70 % B, $t_{3,1}$ = 40 % B and t_6 = 40 % B. The flow rate was 0,7 mL/min and UV detection was performed at 245 nm.

The resulting carrier capacities are presented in Figure 6 and Table 6.

Table 6. Loading of testosterone

System	Carrier capacity / % Stirring 3 days 37°C	stdev	Carrier capacity / % Autoclavation 20 min 125°C	stdev	Relative increase	Increase (%)
1-TES	2.14	0.03	5.74	4.73	2.7	168
2-TES	2.05	0.05	7.28	5.40	3.6	256
4-TES	1.13	0.02	12.14	1.52	10.8	976
5-TES	2.08	0.05	10.42	2.71	5.0	401
6-TES	2.59	0.03	9.81	2.39	3.8	278
7-TES	1.82	0.01	7.58	0.91	4.2	316
9-TES	1.53	0.01	1.86	0.26	1.2	22
Ref-TES	0.36	0.00	0.36	0.01	1.0	-2

where stdev is the standard deviation, and increase and relative increase refer to the increase in carrier capacity by heat treatment over constant temperature stirring.

In Figure 7 the relative increase in carrier capacity by heat treatment loading over constant temperature stirring loading is summarized (data extracted from Examples 2-6).

Example 7 – Effect of heat treatment on loading of octreotide

Octreotide at a concentration of 200 μg/ml was added to 5% amphiphile analogues of loading system 3 (water content 95%). One set of samples were equilibrated at room temperature for 1 day, while another set was autoclaved at 125°C for 20 minutes. The ocreotide concentration was determined by HPLC using a size exclusion column coupled to a UV detector.

The heat treatment increased the portion of carrier-bound drug (Table 6).

Table 6. Effect of heat treatment on carrier-bound fraction of octreotide (loading system 3)

Treatment	% bound octreotide
1 day at room temperature	18.07
After Autoclavation 20 min	30.04

Example 8 – Loading and time effects on dispersion particle size distributions

Loading systems 1-4 generally are very stable to long term storage, and loading with active substance does not

appear to affect this feature. Loading of progesterone
in System 2 (10% by weight per amphiphile) does not
affect the particle size distribution - neither the
loading process itself, nor the stability over three
weeks time (Figures 8 and 9).

Example 9 - Stability of progesterone to heat treatment

The steroid hormone progesterone is dissolved in water
at a level of 1% by weight. The solution is
subsequently heated to 120°C in an autoclave for 20
minutes and cooled to room temperature. The solution is
concentrated by freeze-drying and the residue analysed
for breakdown products by gas chromatography mass
spectrometry.

Example 10 - Higher loading by heat treatment.
Pre-formulations were prepared, including the fatty acid
oleic acid, by the following method.

a)   An initial melt was prepared containing GMO
(85.5%), oleic acid (4.5%) and Lutrol F127 (10%). To 9g
of water under mechanical stirring was added 1g of the
molten mixture to form a coarse dispersion.

b)   The coarse dispersion was homogenised with a
microfluidiser at 345 bar.

c)   The dispersion was heated to 120°C for 20
minutes and cooled to room temperature.

The steroid hormone progesterone was incubated with the

cubic particles formed in steps a) to c), at room
temperature. The equilibrium loading level was 3% by
weight.

The above method was repeated by the active agent
progesterone was included in the aqueous phase prior to
the homogenisation and heat treatment steps. The
loading level was again examined and established to be
18 wt%. The effect upon the particle size distribution
was minimal.

The composition with 18 wt% progesterone generated
above was stored at room temperature for 14 days. No
degradation of the composition or decrease in the
loading level was observed after this time.